08/436265

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9224057.1

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Agent's name GILL JENNINGS & EVERY

Agent's address 53/64 Chancery Lane
London

Postcode WC2A 1HN

Agent's ADP number 745001

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# NOVEL PROTEIN KINASE RECEPTORS

## of the Invention

The present invention relates generally to novel human protein kinase receptors and to the nucleic acid molecules encoding them. More specifically, the invention relates to the isolation of DNA sequences encoding kinase receptors with homology to the transforming growth factor ß type II family of receptors.

## Background to the Invention

The transforming growth factor-beta (TGF-beta) superfamily consists of a family of structurally related proteins, including TGF-betas, activins, inhibins, Mullerian inhibiting substance and bone morphogenic proteins (BMPs), which regulate cell proliferation, differentiation and various other cell functions (for review see Roberts and Sporn, (1990), Peptide Growth Factors and their Receptors, part I, Sporn and Roberts eds Springer-Verlag, Berlin, pp 419-472; Massague (1990), Annu. Rev. Cell Biol. 5, 597-641; Vale et al (1990), Peptide Growth Factors and their Receptors, part I, Sporn and Roberts eds, Springer Verlag, Berlin, pp 419-472; Cate et al, (1990), Peptide Growth Factors and their Receptors, part I, Springer Verlag, Berlin pp 179-210). The action of these growth factors is mediated through binding to specific cell surface receptors. Within this family, TGF-beta receptors have been most thoroughly characterized. By covalently crosslinking radiolabeled TGF-beta to cell surface molecules followed by polyacrylamide gel electrophoresis of the affinity labeled complexes, three distinct size classes of cell surface proteins (in most cases) have been identified, named receptor type I, type II and type III (or betaglycan), (For review see Massague, (1992), Cell 69, 1067-1070). Current evidence suggests that type I and type II receptors are involved in receptor signal transduction (Segarini, (1989) et al. Mol. Endo., 3 261 - 272 Laiho et al (1991), J Biol, Chem. 266 9100-9112), and that the type III receptor (betaglycan) has a more indirect role, possibly by facilitating the binding of ligand to type II receptors (Lin et al (1992) Cell 68 775-785).

Binding analysis with activin A and BMP4 have led to the identification of two coexisting crosslinked affinity complexes of 50-60kDa and 70-80 kDa on responsive cells (Hino et al,

(1989). J Biol. Chem. 264, 10309-10314; Mathews and Vale (1991), Cell 68 775-785; Paralkar et al (1991), Proc. Natl. Acad. Sci. USA, 87 8913-8917). In analogy with TGF-beta receptors they are thought to be signalling receptors and have been named rec type I and type II.

Molecular cloning and sequence determination of the mouse activin receptor II (mActR-II) cDNA (Mathews and Vale, (1991) Cell 65,973-982) revealed the predicted structure of the receptor to be a transmembrane protein with an intracellular serine/threonine kinase domain. The activin receptor is related to the C. elegans daf-1 gene product, the ligand is currently unknown. (Georgi, et al. (1990), Cell 61, 635-645). Recently, ActR-II was shown to be a serine/threonine/tyrosine protein kinase (Nakamura et al (1992), J Biol Chem 267, 18924-18928). Another subtype of the activin receptor, Act-R-IIB of which there are different splicing variants, has recently been cloned, and also appears to code for a receptor-type serine/threonine kinase. (Matthews et al (1992), Science 255, 1702-1705; Attisano et al (1992), Cell 68, 97-108). The TGF-beta type II receptor cDNA was also isolated recently, using an expression cloning strategy, and shown to be a functional transmembrane serine/threonine kinase receptor (Lin et al (1992), Cell 68, 775-785). Although the kinase domains show significant homology, the extracelluar domains of Act-RII, Act-R-IIB and TGF-beta type II show little sequence similarity to each other, although they all contain a cysteine rich region.

To ascertain whether there were other members of this family of receptors, a protocol was designed to clone ActR-II/daf 1 related genes. This strategy made use of the polymerase chain reaction (PCR), using degenerate primers based upon the amino acid sequence similarity between the kinase domains of mAct R.II and daf 1 gene products. This strategy resulted in the isolation of a new family of receptor kinases. These genes showed a 13-39% sequence similarity with ActRII and TGF-beta RII and 40-92% sequence similarity towards each other in the kinase domains.

These cloned genes can be used to express the receptors, which can then be used to isolate the respective ligands. Agonists and antagonists may be designed which may be useful in regulating receptor functions. Antibodies may also be raised against the receptors and used in immunoassays and related analytical methods, to measure and detect the receptors as well as modulate their activity.

## BRIEF DESCRIPTION OF THE FIGURES

**Figure 1** Alignment of the serine/threonine (S/T) kinase domains of related receptors.

**Figure 2** Sequence and characteristics of the primers used in the initial PCR reactions.

**Figure 3** Northern blot analysis of various cell lines - A549 (lanes 1 & 2), HEL without (lane 3) and with TPA treatment (lane 4), HSC-2 (land 6), MCF7 (lane 7), T47D (lanes 8 & 9), LNCAP (lanes 10 & 11). Cells were cultured without serum for lanes 1, 6, 8 and 10. Hybridised with PCR product 11.1 or 11.2.

**Figure 4** Northern blot analysis of various cell lines. HEL and TPA (lane 1), HEL (lane 2), AG1518 (lane 3), PC3U (lane 4). Hybridised with labelled PCR product 11.3.

**Figure 5** Nucleotide sequence and deduced amino acid sequence of ARK-1 (clone HP57).

**Figure 6** Nucleotide sequence and deduced amino acid sequence of ARK1 (clone 3F).

**Figure 7** Nucleotide sequence and deduced amino acid sequence of ARK-2.

**Figure 8** Nucleotide sequence and deduced amino acid sequence of ARK-3.

**Figure 9** Partial nucleotide sequence and deduced amino acid sequence of ARK-4.

**Figure 10** Partial nucleotide sequence and deduced amino acid sequence of ARK-5.

Figure 13 — Partial nucleotide sequence and deduced amino acid sequence of PCR product 3.1.

Figure 14 — Partial nucleotide sequence and deduced amino acid sequ of PCR product 1/2-6.

Figure 15 — Partial nucleotide sequence and deduced amino acid sequence of PCR product 1/2-7.

Figure 16 — Schematic diagram of the ARK-1 gene and protein.

Figure 17 — Schematic diagram of the ARK-2 gene and protein.

Figure 18 — Schematic diagram of the ARK-3 gene and protein.

Figure 19 — Schematic diagram of ARK-4 and ARK-5 gene and protein.

Figure 20 — Sequence alignment between the ARKs.

Figure 21 — Sequence alignment of the cysteine rich domains of the ARKs, TGFßRII, Act-R-II, Act-R-IIB and daf-1 receptors.

Figure 22 — Northern blot analysis of various human tissues hybridised with EMBLA, lane 1 - heart, lane 2 - brain, lane 3 - placenta, lane 4 -lung, lane 5 - liver, lane 6 - skeletal muscle, lane 7 - kidney, lane 8 - pancreas.

**EXAMPLE 1**

To examine the possibility that other receptors for the TGF-beta superfamily possessed sequence similarity, a study was initiated aimed at the cloning of any ActR-II/daf-1 related genes. A polymerase chain reaction (PCR) strategy was designed using degenerate primers based upon the amino acid sequence similarity between the kinase domains of the mActR-II and daf-1 gene product. Figure 1 shows the aligned serine/threonine kinase domains (I-VIII), using the nomenclature of the subdomains according to Hanks et al, (1988), Science 241 42-52 of four related receptors of the TGF-beta superfamily - hTGFBR-II, ActR-IIB, mActR-II and the daf-1 gene product.

Several considerations were applied in the design of the PCR primers. The sequences were taken from regions of homology between the activin receptor II and the daf-1 gene product, with particular emphasis on residues that confer serine/threonine specificity and on residues that are shared by transmembrane kinase proteins and not by cytoplasmic kinases. The primers were designed so that each primer of a PCR set had an approximately similar GC composition, and so that self complementarity and complementarity between the 3' ends of the primer sets were avoided. Degeneracy of the primers was kept as low as possible, in particular avoiding serine, leucine and arginine residues (6 possible codons) and human codon preference was applied. Degeneracy was particularly avoided at the 3' end as unlike the 5' end, where mismatches are tolerated, mismatches at the 3' end dramatically reduce the efficiency of PCR.

In order to facilitate directional subcloning, restriction enzyme sites were included at the 5' end of the primers, with a GC clamp, which permits efficient restrictive enzyme digestion. The primers utilised are shown in Figure 2. The various oligonucleotides were synthesised on an oligonucleotide synthesizer according to the manufacturer's instructions. For the initial PCR reactions, mRNA was isolated from a human erythroleukaemia cell line (HEL 92.1.7) obtained from the America Type Culture Collection (ATCC TIB 180). These cells were chosen as they have been shown to respond to both activin and TGF-beta. Moreover leukaemic cells have proved to be rich sources for the cloning of novel receptor

tyrosine kinases (Partanen et al (1990) Proc. Natl. Acad. Sci. USA 87 8913-8917 and 1992) Mol Cell Biol. 12, 1698-1707). RNA was prepared using the guanidinium isothiocyanate method (Chirgwin, et al, (1979) Biochemistry 18 5294-5299). mRNA was selected using the poly AT tract system (Promega Madison [WI]) as described by the manufacturer's instructions.

The PCR reactions were carried out as follows:- 2$\mu$g of mRNA was reverse transcribed in the presence of 50mM Tris-HCl, pH 8.3, 8mM $MgCl_2$, 30mM KCl, 10mM DTT, 2mM NTPs, excess oligo dT primers and 34 units of AMV reverse transcriptase at 42°C for 2 hours. PCR was performed with a 7.5% aliquot of the reverse transcribed mRNA, in the presence of 50mM KCl, 10mM Tris-HCl pH 8.3, 1.5mM $MgCl_2$, 0.01% gelatin, 0.2mM NTPs, 1$\mu$M of both sense and antisense primers, using 2 units of Taq polymerase. The PCR programme was 5 cycles for 1 minute at 94°C, followed by 1 minute at 50°C, followed by a 2 minute ramp to 72°C, then 1 minute at 72°C. This was followed by 20 cycles of 1 minute at 94°C, followed by 30 seconds at 55°C, followed by 1 minute at 72°C. From the reaction mix a 0.3% aliquant of the material was taken and processed for a second round of PCR amplification. This involved 25 cycles of 1 minute at 94°C, followed by 30 seconds at 55°C followed by 1 minute at 72°C.

General nucleic procedures such as purification of nucleic acids, restriction enzyme digestion, gel electrophoresis, transfer of nucleic acid to solid supports and subcloning were performed essentially according to established procedures as described by Sambrook, Fritsch and Maniatis (1989) Molecular Cloning; a Laboratory Manual. Cold Spring Harbour Laboratory (Cold Spring Harbour, New York, USA).

A sample of the PCR products was analyzed by standard agarose gel electrophoresis for size. Those with a similar size to the relevant sections of the activin receptor II and daf-1 genes (see Table 1), were digested with the relevant restrictive enzymes (BamHI and EcoRI) and subcloned into the pUC plasmid, which had been previously linearised with BamHI and EcoRI. The recombinants were transformed into E. coli. and plated. Individual clones were sequenced using standard double stranded sequencing techniques using the dideoxynucleotide chain termination method as described by Sanger et al; Proc. Nat. Acad. Sci USA 74, 5463-5467, using T7 DNA polymerase.

The sequencing of the inserts revealed that several of the clones that were obtained were derived from ActR-II, ActR-II-ß and TGF-beta RII. However, several of the PCR products were derived from previously unrecognised genes, which showed approximately 35% sequence similarity to Act RII and TGF-beta RII and a 40%-90% sequence similarity towards each other in the kinase domain. A number of the clones derived from the HEL cell line, by PCR are shown in Table 1. Different regions of the kinase domains of the various PCR products were sequenced. For comparison to the percent identity of PCR products with mActR-II and TGF-ßR-II, the sequence identity of the mActR-II and TGF-ß-II for the same region is shown in the sixth column.

Sequence identity is defined as the number of residues that are identical between 2 sequences. Sequence similarity comparisons were performed used the clustal V multiple sequence alignment programme (DNA) star. (Higgens and Sharp (1989) CABIOS 5, 151-153.

**TABLE 1**

| NAME | PRIMERS | INSERT SIZE (in bp) | SIZE OF DNA FRAGMENT IN mActR/hTGFBR IN CLONES (in bp) | SEQUENCE IDENTITY WITH SEQUENCE mActRII/hTGFBRII (in %) | SEQUENCE IDENTITY BETWEEN mActRII AND TGFBR-II (in %) |
|---|---|---|---|---|---|
| 11.1 | B3-S/E8-AS | 460 | 460 | 46/40 | 42 |
| 11.2 | B3-S/E8-AS | 460 | 460 | 49/44 | 47 |
| 11.3 | B3-S/E8-AS | 460 | 460 | 44/36 | 48 |
| 11.29 | B3-S/E8-AS | 460 | 460 | ND/100 | ND |
| 9.2 | B1-S/E8-AS | 800 | 795 | 100/ND | ND |
| 5.1 | B7-S/E8-AS | 140 | 143 | 33/33 | 60 |
| 5.2 | B7-S/E8-AS | 140 | 143 | 40/38 | 60 |
| 3.1 | B7-S/E8-AS | 90 | 143 | 58/67 | 79 |
| 1/2.7 | B7-S/E8-AS | 200 | 143 | 36/35 | 60 |
| 1/2.7 | B7-S/E8-AS | 200 | 143 | 43/37 | 60 |

**EXAMPLE 2**

Northern blots were performed in order to analyse the expression of the different genes to look for interesting expression patterns and also to identify an appropriate source from which to attempt to clone the full length cDNAs.

RNA was prepared from the following cell lines: HEL 92.1.7 (Human Erythroleukaemia), PC-3 (human prostate carcinoma), A549 (human lung adenocarcinoma), T47D and MCF-7 (human breast carcinomas) obtained from the American Type Culture Collection (Rockville MD). PC-3U4 (human prostate carcinoma) obtained from Sten Nilsson at the Department of Oncology, University Hospital, Uppsala, Sweden. HSC-2 (human squamous carcinoma) obtained and isolated as described by Momose et al (1989), J Oral Pathology. Med 18

391-395. AG 1518 (Human foreskin fibriblasts) obtained from the Human Mutant Cell [redacted]itory, Camden New Jersey. LNCAP (Human prostate carcinoma) obtained from the Human Tumour Cell Line Repository, Rye, New York, USA.

10$\mu$g of RNA or 2$\mu$g of mRNA samples were fractionated on agarose gels in the presence of formaldehyde and then transferred onto strengthened nitrocellulose or nylon membranes. The PCR products 11.1, 11.2 and 11.3 were radiolabelled using the random priming method (Feinberg and Vogelstein (1983), Anal Biochemistry 132 6-13) using a multi-prime DNA labelling system and [$\alpha$-$^{32}$P]dCTP. The filters were hybridised with the probes at 42°C over night in 50% formaldehyde, 5 x SSC, 0.1% SDS, 50 mM sodium phosphate and 0.1mg/ml salmon sperm DNA. The filters were washed with 0.5 x SSC,0.1% SDS at 55°C before being exposed to X-ray film.

The results are shown in figures 3 and 4. Genes hybridizing with PCR products 11.1 and 11.2 appear to be ubiquitously expressed, whereas the gene hybridizing with PCR product 11.3 is expressed in fibroblasts (figure 4) whereas expression was not detected in A549, HEL, HSC-2, MCF-7, T47D and LNCAP cells (data not shown).

**EXAMPLE 3**

In order to obtain full length clones for the various PCR products, the PCR probes were used to screen various cDNA libraries. The clones were isolated from the libraries using standard molecular biological techniques. The human placental lambda ZAPII DNA library was obtained from Hideo Toyoshima, University of Tokyo, Japan. (cDNA clones HP22, HP57, HP29, HP53 and HP83 were obtained from this library). The human fibroblast AG1518 lambda ZAPII, cDNA and HEL cell lambda gt10 cDNA libraries were prepared with

Ribo clone cDNA synthesis system (Promega) and Giga pack Gold (Stratagene) and lambda gt10 in vitro packaging kit (Amersham) respectively, according to the manufactu[illegible] procedures, cDNA clones ONF1, ONF2, ONF3, ONF4 and ONF5 were obtained from [illegible] human fibroblast library. The cDNA clone EMBLA was obtained from the HEL lambda lgt10 cDNA library. The human fibroblast AG1518 lambda gt10 library was obtained from Lena Cleasson-Welsh as described in Cleasson-Welsh et al. (1989), Proc. Nat. Acad. Sci. USA 86 4917-4921. The cDNA clones ON and 3F were obtained from this library. The HEL cell lambda gt11 cDNA library was obtained from Mortimer Poncz and was described in Poncz et al (1987) Blood 69 219-223. The cDNA clone 11H8 was obtained from this library.

The double stranded DNA clones were all sequenced using the dideoxynucleotide chain-termination method as described by Sanger et al (1977), Proc. Nat. Acad. of Sci. USA 74 5463 - 5467, using T7 DNA polymerase.

Analysis of the sequences obtained revealed the existence of five distinct putative receptor serine/threonine kinases which have been named ARK1-5 (Activin-receptor Related Kinases). The relation of the cDNA clones to each other is shown below.

To summarise clones 3F, HP22, HP57, ONF1, ONF3, ONF4 and HP29 encode the same gene ARK-1. HP53, HP64 and HP84 encode the same gene ARK-2. ONF5, ONF2 and ON11 encode the same gene ARK3. 11H8 encodes a different gene ARK-4. EMBLA encodes ARK-5.

Two of the clones which encode the ARK-1 gene, HP57 and 3F have been sequenced. These are shown in figures 5 and 6, respectively. These were both obtained by screening

the human placental cDNA library with PCR product 11.3 as a probe. Although, the clones appear to be derived from the same gene, the clones vary in the 5' and 3' untranslated sequences as well as in the C terminal tail (C terminal of the kinase domain). The difference in the 5' untranslated sequence could be explained by alternative processing or the use of different promoters. The 3' end of the HP57 clones contains a poly A tract, as found in most cDNA's. However, a poly T tract is found in the 3' end of the 3F clone which is probably a cDNA cloning artifact. The predicted C-termini of 3F and HP57 are different due to the addition of one nucleotide (A) in the 3F sequence, as compared to the HP57 sequence (nucleotide position 1755 in 3F).

The complete sequence of the cDNA clone HP53 is shown in figure 7. This clone was obtained by screening the human placental cDNA library with PCR product 11.2 as a probe. This sequence has been named activin related kinase-2 (ARK 2).

The activin receptor related kinase - 3 (ARK-3) sequence is shown in figure 8. This is the sequence of clone ONF5 which was obtained by screening the human foreskin fibroblast cDNA library described, with the kinase domain of cDNA clone ON11 as a probe. cDNA clone ON11 was obtained from the human fibroblast cDNA library using an oligonucleotide derived from PCR product 5.2, as a probe.

The partial amino acid sequence of the activin receptor related kinase-4 (ARK-4) is shown in figure 9. This is deduced from the nucleotide sequence of PCR product 11.1 obtained from human erythroleukaemia cell mRNA, using primers B3-S and E8-AS in the PCR, as described previously. A clone has been isolated from the HEL library using the PCR product 11.1 as a probe and will be further characterised.

The nucleotide sequence and deduced amino acid sequence of the activin receptor [illegible]ted kinase-5 (ARK-5) is shown in figure 10. This is the sequence of the cDNA clone [illegible]LA which was obtained by screening the HEL cDNA library with PCR products 11.1 and 11.2 as a probe.

The sequence similarity between the five ARK genes and TGFßRII, ActR-IIB and mAct-RII in the serine/threonine kinase domains is shown in figure 11. The five members of this family have a 40-49% sequence similarity in the serine/thronine kinase domain with approximately 35% sequence similarity to the same domain in the Activin and TGF-beta RII receptors. The increased homology between the ARK receptors as compared to the TGFßRII and the activin receptors places them in a distinct family.

Further novel sequences have been obtained from other PCR products. Figures 12-15 show the nucleotide sequences and deduced amino acid sequences of a series of PCR products arising from a reaction using human erythroleukaemia cells as the mRNA source in the cDNA preparation and primers B7-S and E8-AS as described supra. The primer sequences are included and these sequences, in particular those forming the restriction sites, may not correspond to the actual gene sequence from which the PCR product was derived. These partial cDNA sequences can be used to isolate the full length sequences from the cDNA libraries described above.

Sequence analysis of the cDNAs for this family of genes revealed that they code for proteins with a ligand binding extracelluar domain with a characteristic cysteine rich region, a single hydrophobic membrane spanning domain and an intracellular kinase domain with a predicted serine/threonine specificity. The recent finding that the of ActR-II receptor has serine/threonine/tyrosine kinase activity (Nakamura et al (1992), J Biol. Chem.

267, 18924-18928), suggest that these kinases may also have tyrosine kinase activity.

Schematic diagrams of each of the five genes described, are shown in figures 16-19. A sequence alignment between the genes is shown in figure 20. Alignment between the cysteine rich domains of the ARK cDNAs is shown in figure 21. The homology is low, approximately 10-20%, however, the positions of the cysteine residues are conserved, suggesting that the receptors probably bind different but related ligands.

Further Northern analysis was performed to study the expression of ARK-5 in various human tissues. Figure 21 shows that ARK-5 appears to be ubiquitously expressed as a 5.5kb message in various tissues. Expression is particularly high in the brain and skeletal muscle.

The foregoing describes the isolation of nucleic acid sequences coding for a new family of human receptor kinases. The term nucleic acid molecules as used herein refers to any sequence which code for the human form, amino acid sequences of which are presented herein. It is understood that the well known phenomenon of codon degeneracy provides for a great deal of sequence variation and all such varieties are included within the scope of this invention. The nucleic acid sequence described in the application may be used to clone the respective genomic DNA sequences in order to study the genes' structure and regulation. The human cDNA or genomic sequences can also be used to isolate the homologous genes from other mammalian species. The mammalian DNA sequences can be used to study the receptors' functions in various in vitro and in vivo model systems.

It is also to be recognized that given the sequence information provided herein, the artisan could easily combine the molecules with a pertinent promoter in a vector, so as to produce

a cloning vehicle. The promoter and coding molecule must be operably linked via one of the well recognized and easily practised methodologies for so doing. The resulting vectors, as well as the isolated nucleic acid molecules themselves, may be used to transfect cells, such as the prokaryotic cells (eg, E. coli), or eukaryotes such as yeast (S. cervisae), COS or CHO cell lines. Other recipient cells will also be apparent to the skilled artisan.

Several methods may be used to isolate the ligands for the ARKs. cDNA clones encoding the active open reading frames can be subcloned into expression vectors and transfected into eukaryotic cells, for example COS cells. The transfected cells which can express the receptor will be subjected to binding assays for radioactively labelled members of the TGF-beta superfamily (TGF-beta, activins, inhibins, bone morphogenic proteins and mullerian inhibiting substance), as it might be predicted that the receptors will bind members of the TGF-beta superfamily. Various biochemical or cell based assays can be designed to identify the ligands, in tissue extracts or conditioned media, for receptors for which a ligand is not found. Alternatively, purified receptor could be used to isolate the ligands using an affinity based approach. The determination of the expression patterns of the receptors may also aid in the isolation of the ligand. These studies may be carried out using the ARK DNA or RNA sequences to perform in situ hybridisation studies. Antibodies raised to the receptors may also be used to perform this analysis.

The use of various model systems or structural studies should enable the development of specific agonists and antagonists useful in regulating receptor function. This molecules would either mimic the effect of the respective ligand (agonists) or block the effect of the ligand (antagonists). It may be envisaged that these can be amino acid containing molecules, antibodies or other molecules able to interact with the receptors.

The foregoing constitutes examples of the invention applicants intend to claim which includes interalia, isolated nucleic acid moleculas coding for Activin receptor Related kinases (ARKs), as defined herein. These include such sequences isolated from mammalia species such as human, mouse, rat, rabbit and monkey.

It will be understood that the specification and examples are illustrative but not limitative of the present invention and that other embodiments within the spirit and scope of the invention will suggest themselves to those skilled in the art.

Figure 1

S/T kinase domains (I to VIII) from transmembrane proteins

```
cons.aa          G G  G V                A K          E
hTGFBR-II   LDTLVGKGRFAEVYKAKLKQNTSEQFETVAVKIFPYDHYASWKDRKDIFSDINLKHENILQF
mActR-IIB   LLEIKARGRFGCVWKAQLMN------DFVAVKIKPLQDKQSWQSEREIFSTPGMKHENLLQF
mActR-II    LLEVKARGRFGCVWKAQLLN------EYVAVKIFPIQDKQSWQNEYEVYSIPGMKHENILQF
daf-1       LTGRVGSGRFGNVSRGDYRG------EAVAVKVFNAIDEPAFHKEIEIFETRMLRHPNVLRY
subdomains         I                          II        III          IV

hTGFBR-II   LTAEERKTELGKQYWLITAFHAKGNLQEYLTRHVISWEDLRNVGSSLARGLSHLHSDHTP-C
mActR-IIB   IAAEKRGSNLEVELWLITAFHDKGSLIDYLKGNIITWNELCHVAETMSRGISYLHEDVPWCR
mActR-II    IGAEKRGTSVDVDLWLITAFHEKGSLSDFLKANVVSWNELCHIAETMARGLAYLHEDIPGLK
daf-1       IGSDRVDTGFVTELWLVIEYHPSGSLHDFLLENTVNIETYYNLMRSTASGLAFLHNQIGGSK
subdomains                             V                        VI-A

cons.aa               DLK  N              DFG                          ____
hTGFBR-II   -GRPKMPIVHRDLKSSNILVKNDLTCCLCDFGLSLRL---GPYSSVDDLANSGQVGTARYMAP
mActR-IIB   GEGHKPSIAHRDFKSKNVLLKSDLTAVLADFGLAVRF---EPGKPPGD--THGQVGTRRYMAP
mActR-II    -DGHKPAISHRDIKSKNVLLKNNLTACIADFGLALKF---EAGKSAGD--THGQVGTRRYMAP
daf-1       -ESNKPAMAHRDIKSKNIMYKNDLTCAIGDLGLSLSKPEDAASDIIAN--ENYKCGTVRYLAP
subdomains       VI-B                          VII                    VIII
```

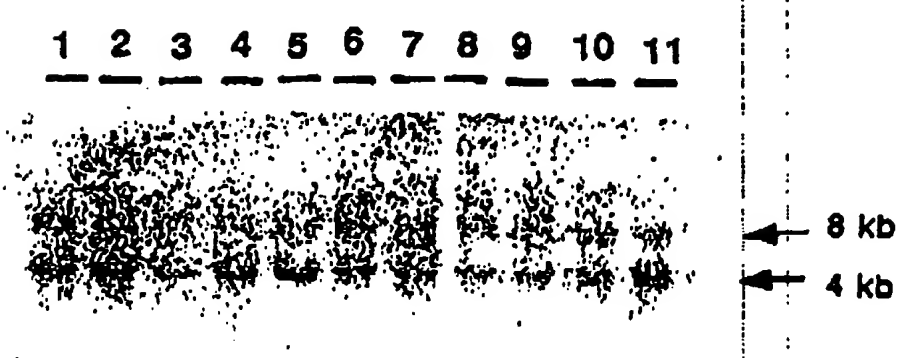
(nomenclature of the subdomains according to Hanks et al, 1988)

Fig. 2

**PCR primers**

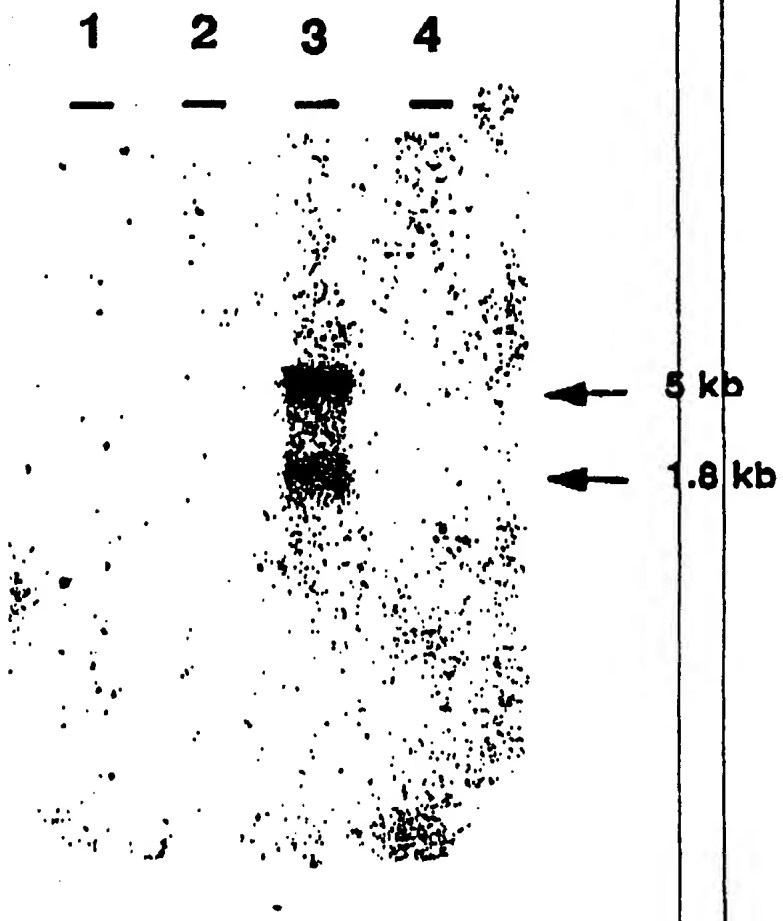
| Name | Sequence and characteristics |
|---|---|
| B1-S | sense primer, extracellular domain, cysteine rich region, BamHI site at 5' end, 28 mer, 64 fold degeneracy<br>a.a C C E G N M C<br>5' GCGGATCCTGTTGTGAAGGNAATATGTG 3'<br>BAMHI C C G C |
| B2-S | sense primer, kinase domain I<br>BamHI site at 5' end, 25 mer, 384 fold degeneracy<br>a.a G R F G C V<br>5' GCGGATCCGGNCGCTTTGGNTGTGT 3'<br>BamHI A A C C<br>G |
| B3-S | sense primer, kinase domain II<br>BamHI site at 5' end , 25 mer, 162 fold degeneracy<br>a.a V A V K I F<br>5' GCGGATCCGTCGCAGTCAAAATTTT 3'<br>BamHI G C G G C<br>T T T A |
| B7-S | sense primer, kinase domain VIB, S/T kinase specific residues<br>BamHI site at 5' end, 24 mer, 288 fold degeneracy<br>a.a R D I K S K N<br>5' GCGGATCCGCGATATTAAAAGCAA 3'<br>BAMHI A C C GTCT<br>G A |
| E8-AS | anti sense primer, kinase domain, S/T kinase specific residues<br>EcoRI site at 5' end, 20 mer, 18 fold degeneracy<br>a.a E P A M Y<br>5' CGGAATTCTGGTGCCATATA<br>EcoRI G G G<br>A A |

Figure3




Northern blot analysis
Total RNA (10 microgram) from A549 (lanes 1 and 2), HEL without (lane 3) and HEL with TPA treatment,1.6 microM for 24 hr's (lane 4), HSC-2, MCF-7 (lanes 6 and 7), T47D (lanes 8 and 9), LNCAP without (lane10) and with treatment of synthetic androgen R1881 (300 microgram/ml) for 48 hr's., lane11). RNA from cells in lanes 2, 6, and 9 were cultured without serum addition to the media. RNA from cells in lanes 1, 3, 4, 5, 7, 8, 10 and 11 were cultured in the presence of serum. Upon fractionation the RNA was transferred to Hybond N and hybridized with radiolabeled PCR product 11.1 or 11.2 at 42 C in the presence of 50 % formamide and 5 x SSC (both probes resulted in identical pattern). Filter was washed with 0.5 x SSC, 0.1 % SDS at 55 C.

Figure 4.




Northern blot analysis
RNA from HEL treated with TPA (lane 1; 2 microgram mRNA), HEL (lane2 ; 2 microgram mRNA), AG1518 (lane 3 ; 2 microgram mRNA) and PC3U (lane 4 ; 10 microgram total RNA) was transferred to Hybond C-extra and hybridized with radiolabeled PCR product 11.3 at 42 C in the presence of 50 % formamide and 5 x SSC. Filter was washed with 0.5 x SSC, 0.1 % SDS at 55 C.

ARK-1 HP67 Figure 5

(2)

```
GAATTCGGCGGCCGCGGATCCAAGGAAACGGTTTATTAGGAGGGAGTGGTGGAGCTGGGCCAGGCAGGAA                    70
GACGCTGGAATAAGAAACATTTTTTCTCCAGCCCCCATCCCAGTCCCGGGAGGCTGCCGCGCCAGCTGCG                   140
CCGAGCGAGCCCCTCCCCGGCTCCAGCCCGGTCCGGGGCCGCGCCGGACCCCAGCCCGCCGTCCAGCGCT                   210
GGCGGTGCAACTGCGGCCGCGCGGTGGAGGGCAGGTGGCCCCCGGTCCGCCGAACGTAGCGCCCCGCCACC                   280
CGCAGACCGGGCCCAGAGGGACCATGACCTTGGGCTCCCCCAGGAAAGGCCTTCTGATGCTGCTGATGGC                    350
                                M  T  L  G  S  P  R  K  G  L  L  M  L  L  M  A
CTTGGTGACCCAGCGAGACCCTGTGAAGCCGTCTCGGGGCCCGCTGGTGACCTGCACGTGTGAGAGCCCA                   420
   L  Y  T  G  G  D  P  V  K  P  S  R  G  P  L  V  T  C  T  C  E  S  P
CATTGCAAGGGGCCTACCTGCCGGGGGGCCTGGTGCACAGTAGTGCTGGTGCGGGAGGAGGCGAGGCACC                   490
 H  C  K  G  P  T  C  R  G  A  W  C  T  V  V  L  V  R  E  E  E  R  H
CCCAGGAACATCGGGGCTGCGGGAACTTGCACAGGGAGCTCTGCAGGGGCGCCCCACCGAGTTCGTCAA                    560
P  Q  E  H  R  G  C  G  N  L  H  R  E  L  C  R  G  R  P  T  E  F  V  N
CCACTACTGCTGCGACAGCCACCTCTGCAACCACAACGTGTCCCTGGTGCTGGAGGCCAGCCAACCTCCT                   630
  H  Y  C  C  D  S  H  L  C  N  H  N  V  S  L  V  L  E  A  T  Q  P  P
TCGGACCAGCCGGGAACAGATGGCCAGCTGGCCCTGATCCTGGGCCCCGTGCTGGCCTTGCTGGCCCTGG                   700
 S  E  Q  P  G  T  D  G  Q  L  A  L  I  L  G  P  V  L  A  L  L  A  L
TGGCCCTGGGTGTCCTGGGCCTGTGGCATGTCCGACGGAGGCAGGAGAAGCAGCGTGGCCTGCACAGCGA                   770
V  A  L  G  V  L  G  L  W  H  Y  R  R  R  Q  E  K  Q  R  G  L  H  S  E
GCTGGCAGAGTCCAGTCTCATCCTGAAAGCATCTGAGCAGGGCGACACGATGTTGGGGGACCTCCTGGAC                   840
  L  G  E  S  S  L  I  L  K  A  S  E  Q  G  D  T  M  L  G  D  L  L  D
AGTGACTGCACCACACGGAAGTGGCTCAGGGCTCCCCTTCCTGGTGCAGAGGACAGTGGCACGGCAGGTTG                  910
 S  D  C  T  T  G  S  G  S  G  L  P  F  L  V  Q  R  T  V  A  R  Q  V
CCTTGGTGGAGTGTGTGGCAAAAGGCCGCTATGGCGAAGTGTGGCGGGGCTTGTGGCACGGTGAGAGTGT                  980
A  L  V  E  C  V  G  K  G  R  Y  G  E  V  W  R  G  L  W  H  G  E  S  V
GGCCGTCAAGATCTTCTCCTCGAGGGATGAACAGTCCTGGTTCCGGGAGACTGAGATCTATAACACAGTA                  1050
  A  V  K  I  F  S  S  R  D  E  Q  S  W  F  R  E  T  E  I  Y  N  T  V
TTGCTCAGACACGACAACATCCTAGGCTTCATCGCCTCAGACATGACCTCCCGCAACTCGAGCACGCAGC                  1120
 L  L  R  H  D  N  I  L  G  F  I  A  S  D  M  T  S  R  N  S  S  T  Q
TGTGGCTCATCACGCACTACCACGAGCACGGCTCCCTCTACGACTTTCTGCAGAGACAGACGCTGGAGCC                  1190
L  W  L  I  T  H  Y  H  E  H  G  S  L  Y  D  F  L  Q  R  Q  T  L  E  P
CCATCTGGCTCTGAGGCTAGCTGTGTCCGCGGCATGCGGCCTGGCGCACCTGCACGTGGAGATCTTCGGT                  1260
  H  L  A  L  R  L  A  V  S  A  A  C  G  L  A  H  L  H  V  E  I  F  G
ACACAGGGCAAACCAGCCATTGCCCACCGCGACTTCAAGAGCCGCAATGTGCTGGTCAAGAGCAACCTGC                  1330
 T  G  G  K  P  A  I  A  H  R  D  F  K  S  R  N  V  L  V  K  S  N  L
AGTGTTGCATCGCCGACCTGGGCCTGGCTGTGATGCACTCACAGGGCAGCGATTACCTGGACATCGGCAA                  1400
 Q  C  C  I  A  D  L  G  L  A  V  M  H  S  Q  G  S  D  Y  L  D  I  G  N
CAACCCGAGAGTCGGCACCAAGCGGTACATGGCACCCGAGGTGCTGGACGAGCAGATCCGCACCGACTGC                  1470
  N  P  R  V  G  T  K  R  Y  M  A  P  E  V  L  D  E  Q  I  R  T  D  C
TTTGAGTCCTACAAGTGGACTGACATCTGGGCCTTTGGCCTGGTGCTGTGGGAGATTGCCCGCCGGACCA                  1540
 F  E  S  Y  K  W  T  D  I  W  A  F  G  L  V  L  W  E  I  A  R  R  T
```

HP 57

(3)

```
TCGTGAATGGCATCGTGGAGGACTATAGACCACCCTTCTATGATGTGGTGCCCAATGACCCCAGGTTTGA
                                                                      1610
I  V  N  G  I  V  E  D  Y  R  P  P  F  Y  D  V  V  P  N  D  P  S  F  E
GGACATGAAGAAGGTGGTGTGTGTGGATCAGCAGACCCCCACCATCCCTAACCGGCTGGCTGCAGACCCG
                                                                      1680
  D  M  K  K  V  V  C  V  D  Q  Q  T  P  T  I  P  N  R  L  A  A  D  P
GTCCTCTCAGGCCTAGCTCAGATGATGCGGGAGTGCTGGTACCCAAACCCCTCTGCCCGACTCAGCGCGC
                                                                      1750
  Y  L  S  G  L  A  Q  M  M  R  E  C  W  Y  P  N  P  S  A  R  L  T  A
TGCGGATCAAGAAGACACTACAAAAAATTAGCAACAGTCCAGAGAAGCCTAAAGTGATTCAATAGCCCAG
                                                                      1820
L  R  E  K  K  T  L  Q  K  I  S  N  S  P  E  K  P  K  V  I  Q  .
GAGCACCTGATTCCTTTCTGCCTGCAGGGGGCTGGGGGGGTGGGGGGCAGTGGATGGTGCCCTATCTGGG
                                                                      1890
TAGAGGTAGTGTGAGTGTGGTGTGTGCTGGGGATGGGCAGCTGCGCCTGCCTGCTCGGCCCCCAGCCCAC
                                                                      1960
CCAGCCAAAAATACAGCTGGGCTGAAACCTGAAAAAAAAAAAAAA
                                                                      2005
```

Nucleotide sequence and deduced amino acid sequence of activin receptor related kinase - 1 (ARK-1). The complete sequence of cDNA cloneHP57 is shown, which was obtained by screening a human placenta cDNA library with PCR product 11.3 as a probe. cDNA clones HP57 and 3F appear to be derived from the same gene ; both clones differ in the 5' and 3' untranslated sequence as well as in the C terminal tail (C-terminal of the kinase domain).

ARK-1 (3F) Figure 6

(4)

```
GAATTCGGCACGAGGCTAGCCCCAAACTCTGCCCTCATTGCTGCAAGCCTCCTAGACCGAGGACCCCCGG                70
NCTGAGGGTGGGCCCCCATCCCAGTCCCGGGAGGCTGCCGCGCCAGCTGCGCCGAGCGAGCCCCTCCCCG              140
GCTCCAGCCCGGTCCGGGGCCGCGCCGGACCCCAGCCCGCCGTCCAGCGCTGGCGGTGCAACTGCGGCCG              210
CGCGGTGGAGGGGAGGTGGCCCCGGTCCGCCGAACGTAGCGCCCCGCCACCCGCAGAGCGGGCCCAGAGG              280
GACCATGACCTTGGGCTCCCCCAGGAAAGGCCTTCTGATGCTGCTGATGGCCTTGGTGACCCAGGGAGAC              350
                M  T  L  G  S  P  R  K  G  L  L  M  L  L  M  A  L  V  T  Q  G  D
CCTGTGAAGCCGTCTCGGGGCCCGCTGGTGACCTGCACGTGTGAGAGCCCACATTGCAAGGGGCCTACCT              420
P  V  K  P  S  R  G  P  L  V  T  C  T  C  E  S  P  H  C  K  G  P  T
GCCCGGCGGCCCTGGTGCACAGTAGTGCTGGTCCGGGAGGAGGGGAGGCACCCCCAGGAACATCGGGGCTG              490
C  R  G  A  W  C  T  V  V  L  V  R  E  E  G  R  H  P  Q  E  H  R  G  C
CGGGAACTTGCACAGGGAGCTCTGCAGGGGGCGCCCCACCGAGTTCGTCAACCACTACTGCTGCGACAGC              560
Q  N  L  H  R  E  L  C  R  G  R  P  T  E  F  V  N  H  Y  C  C  D  S
CACGTCTGCAACCACAACGTGTCCCTGGTGCTGGAGGCCACCCAACCTCCTTCGGAGCAGCCGGGAACAG              630
H  L  C  N  H  N  V  S  L  V  L  E  A  T  Q  P  P  S  E  Q  P  G  T
ATGCCCAGCTGGCCCTGATCCTGGGCCCCGTGCTGGCCTTGCTGGCCCTGGTGGCCCTGGGTGTCCTGGG              700
D  G  Q  L  A  L  I  L  G  P  V  L  A  L  L  A  L  V  A  L  G  V  L  G
CCTGTGGCATGTCCGACGGAGGCAGGAGAAGCAGCGTGGCCTGCACAGCGAGCTGGGAGAGTCCAGTCTC              770
L  W  H  V  R  R  R  Q  E  K  Q  R  G  L  H  S  E  L  G  E  S  S  L
ATCCTGAAAGCATCTGAGCAGGGCGACACGATGTTGGGGGACCTCCTGGACAGTGACTGCACCACAGGGA              840
I  L  K  A  S  E  Q  G  D  T  M  L  G  D  L  L  D  S  D  C  T  T  G
CTGGCTCAGGGCTCCCCTTCCTGGTGCAGAGGACAGTGGCACGGCAGGTTGCCTTGGTGCAGTGTGTGGG              910
S  G  S  G  L  P  F  L  V  Q  R  T  V  A  R  Q  V  A  L  V  E  C  V  G
AAAAGGCCGCTATGGCGAAGTGTGGCGGGGCTTGTGGCACGGTGAGAGTGTGGCCGTCAAGATCTTCTCC              980
K  G  R  Y  G  E  V  W  R  G  L  W  H  G  E  S  V  A  V  K  I  F  S
TCGAGGGATGAACAGTCCTGGTTCCGGGAGACTGAGATCTATAACACAGTATTGCTCAGACACGACAACA             1050
S  R  D  E  Q  S  W  F  R  E  T  E  I  Y  N  T  V  L  L  R  H  D  N
TCCTAGGCTTCATCGCCTCAGACATGACCTCCCGCAACTCGAGCACGCAGCTGTGGCTCATCACGCACTA*            1120
I  L  G  F  I  A  S  D  M  T  S  R  N  S  S  T  Q  L  W  L  I  T  H  Y
CGACGAGCACGGCTCCCTCTACGACTTTCTGCAGAGACAGACGCTGGAGCCCCATCTGGCTGTGAGGCTA             1190
H  E  H  G  S  L  Y  D  F  L  Q  R  Q  T  L  E  P  H  L  A  L  R  L
GCTGTGTCCGCGGCATGCGGCCTGGCGCACCTGCACGTGGAGATCTTCGGTACACAGGGCAAACCAGCCA             1260
A  V  S  A  A  C  G  L  A  H  L  H  V  E  I  F  G  T  Q  G  K  P  A
TTGCCCACCGCGACTTCAAGAGCCGCAATGTGCTGGTCAAGAGCAACCTGCAGTGTTGCATCGCCGACCT             1330
I  A  H  R  D  F  K  S  R  N  V  L  V  K  S  N  L  Q  C  C  I  A  D  L
GGGCCTGGCTGTGATGCACTCACAGGGCAGCGATTACCTGGACATCGGCAACAACCCGAGAGTGGGCACC             1400
G  L  A  V  M  H  S  Q  G  S  D  Y  L  D  I  G  N  N  P  R  V  G  T
AAGCGGTACATGGCACCCGAGGTGCTGGACGAGCAGATCCGCACGGACTGCTTTGAGTCCTACAAGTGGA             1470
K  R  Y  M  A  P  E  V  L  D  E  Q  I  R  T  D  C  F  E  S  Y  K  W
CTCACATCTCGGCCTTTGGCCTGGTGCTGTGGGAGATTGCCCGCCGGACCATCGTGAATGGCATCGTGGA             1540
T  D  I  W  A  F  G  L  V  L  W  E  I  A  R  R  T  I  V  N  G  I  V  E
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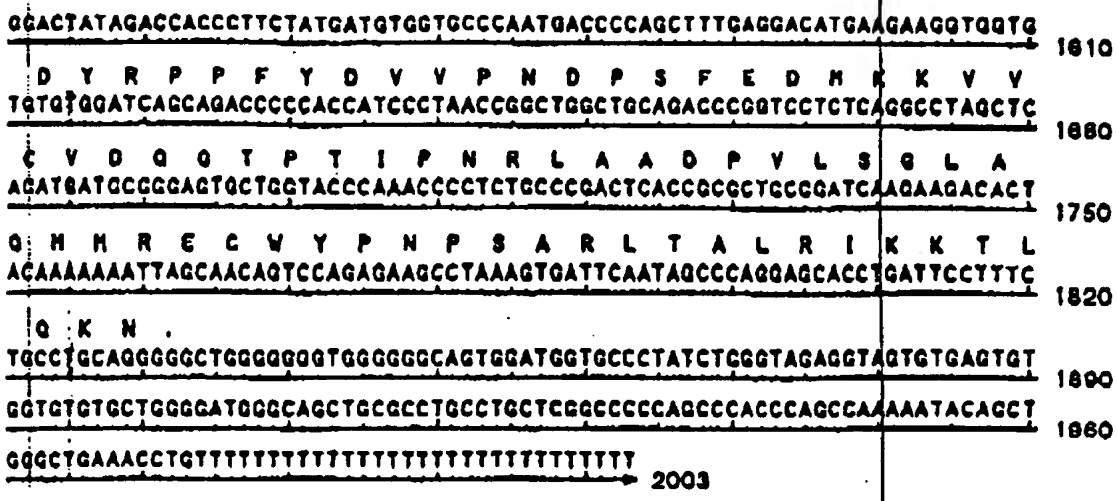
(3F)

(5)




Nucleotide sequence and deduced amino acid sequence of activin receptor related kinase - 1 (ARK-1). The complete sequence of cDNA clone3F is shown, which was obtained by screening a human foreskin fibroblast cDNA library with PCR product 11.3 as a probe. cDNA clones 3F and HP57 appear to be derived from the same gene ; both clones differ in the 5' and 3' untranslated sequence as well as in the C terminal tail (C-terminal of the kinase domain).

AKR-2 Sequence Figure 7

(2)

```
CTCCGAGTACCCCAGTGACCAGAGTGAGAGAAGCTCTGAACGAGGGCACGCGGCTTGAAGGACTGTGGGC
AGATGTGACCAAGAGCCTGCATTAAGTTGTACAATGGTAGATGGAGTGATGATTCTTCCTGTGCTTATCA
                                                                        70
                                                                       140
                                  M  V  D  G  V  M  I  L  P  V  L  I
TGATTGCTCTCCCCTCCCCTAGTATGGAAGATGAGAAGCCCAAGGTCAACCCCAAACTCTACATGTGTGT
                                                                       210
M  I  A  L  P  S  P  S  M  E  D  E  K  P  K  V  N  P  K  L  Y  M  C  V
GTGTGAAGGTCTCTCCTGCGGTAATGAGGACCACTGTGAAGGCCAGCAGTGCTTTTCCTCACTGAGCATC
                                                                       280
  C  E  G  L  S  C  G  N  E  D  H  C  E  G  Q  Q  C  F  S  S  L  S  I
AACGATGGCTTCCACGTCTACCAGAAAGGCTGCTTCCAGGTTTATGAGCAGGGAAAGATGACCTGTAAGA
                                                                       350
  N  D  G  F  H  V  Y  Q  K  G  C  F  Q  V  Y  E  Q  G  K  M  T  C  K
CCCCGCCGTCCCCTGGCCAAGCTGTGGAGTGCTGCCAAGGGGACTGGTGTAACAGGAACATCACGGCCCA
                                                                       420
T  P  P  S  P  G  Q  A  V  E  C  C  Q  G  D  W  C  N  R  N  I  T  A  Q
GCTGCCCACTAAAGGAAAATCCTTCCCTGGAACACAGAATTTCCACTTGGAGGTTGGCCTCATTATTCTC
                                                                       490
  L  P  T  K  G  K  S  F  P  G  T  Q  N  F  H  L  E  V  G  L  I  I  L
TCTGTAGTGTTYGCAGTATGTCTTTTAGCCTGCCTGCTGGGAGTTGCTCTCCGAAAATTTAAAAGGCGCA
                                                                       560
  S  V  V  F  A  V  C  L  L  A  C  L  L  G  V  A  L  R  K  F  K  R  R
ACCAAGAACGCCTCAATCCCCGAGACGTGGAGTATGGCACTATCGAAGGGCTCATCACCACCAATGTTGG
                                                                       630
N  Q  E  R  L  N  P  R  D  V  E  Y  G  T  I  E  G  L  I  T  T  N  V  G
AGACAGCACTTTAGCAGATTTATTGGATCATTCGTGTACATCAGGAAGTGGCTCTGGTCTTCCTTTTCTG
                                                                       700
  D  S  T  L  A  D  L  L  D  H  S  C  T  S  G  S  G  S  G  L  P  F  L
GTACAAAGAACAGTGGCTCGCCAGATTACACTGTTGGAGTGTGTCGGGAAAGGCAGGTATGGTGAGGTGT
                                                                       770
  V  Q  R  T  V  A  R  Q  I  T  L  L  E  C  V  G  K  G  R  Y  G  E  V
GGAGGGGCAGCTGGCAAGGGGAAAATGTTGCCGTGAAGATCTTCTCCTCCCGTGATGAGAAGTCATGGTT
                                                                       840
W  R  G  S  W  Q  G  E  N  V  A  V  K  I  F  S  S  R  D  E  K  S  W  F
CAGGGAAACGGAATTGTACAACACTGTGATGCTGAGGCATGAAAATATCTTAGGTTTCATTGCTTCAGAC
                                                                       910
  R  E  T  E  L  Y  N  T  V  M  L  R  H  E  N  I  L  G  F  I  A  S  D
ATGACATCAAGACACTCCAGTACCCAGCTGTGGTTAATTACACATTATCATGAAATGGGATCGTTGTACG
                                                                       980
M  T  S  R  H  S  S  T  Q  L  W  L  I  T  H  Y  H  E  M  G  S  L  Y
ACTATCTTCAGCTTACTACTCTGGATACAGTTAGCTGCCTTCGAATAGTGCTGTCCATAGCTAGTGGTCT
                                                                      1050
D  Y  L  Q  L  T  T  L  D  T  V  S  C  L  R  I  V  L  S  I  A  S  G  L
TGCACATTTGCACATAGAGATATTTGGGACCCAAGGGAAACCAGCCATTGCCCATCGAGATTTAAAGAGC
                                                                      1120
  A  H  L  H  I  E  I  F  G  T  Q  G  K  P  A  I  A  H  R  D  L  K  S
AAAAATATTCTGGTTAAGAAGAATGGACAGTGTTGCATAGCAGATTTGGGCCTGGCAGTCATGCATTCCC
                                                                      1190
  K  N  I  L  V  K  K  N  G  Q  C  C  I  A  D  L  G  L  A  V  M  H  S
AGAGCACCAATCAGCTTGATGTGGGGAACAATCCCCGTGTGGGCACCAAGCGCTACATGGCCCCCGAAGT
                                                                      1260
Q  S  T  N  Q  L  D  V  G  N  N  P  R  V  G  T  K  R  Y  M  A  P  E  V
TCTAGATGAAACCATCCAGGTGGATTGTTTCGATTCTTATAAAAGGGTCGATATTTGGGCCTTTGCACTT
                                                                      1330
  L  D  E  T  I  Q  V  D  C  F  D  S  Y  K  R  V  D  I  W  A  F  G  L
GTTTTGTGGGAAGTGGCCAGGCGGATGGTGAGCAATGGTATAGTGGAGGATTACAAGCCACCGTTCTACG
                                                                      1400
  V  L  W  E  V  A  R  R  M  V  S  N  G  I  V  E  D  Y  K  P  P  F  Y
ATGTGGTTCCCAATGACCCAAGTTTTGAAGATATGAGGAAGGTAGTCTGTGTGGATCAACAAAGGCCAAA
                                                                      1470
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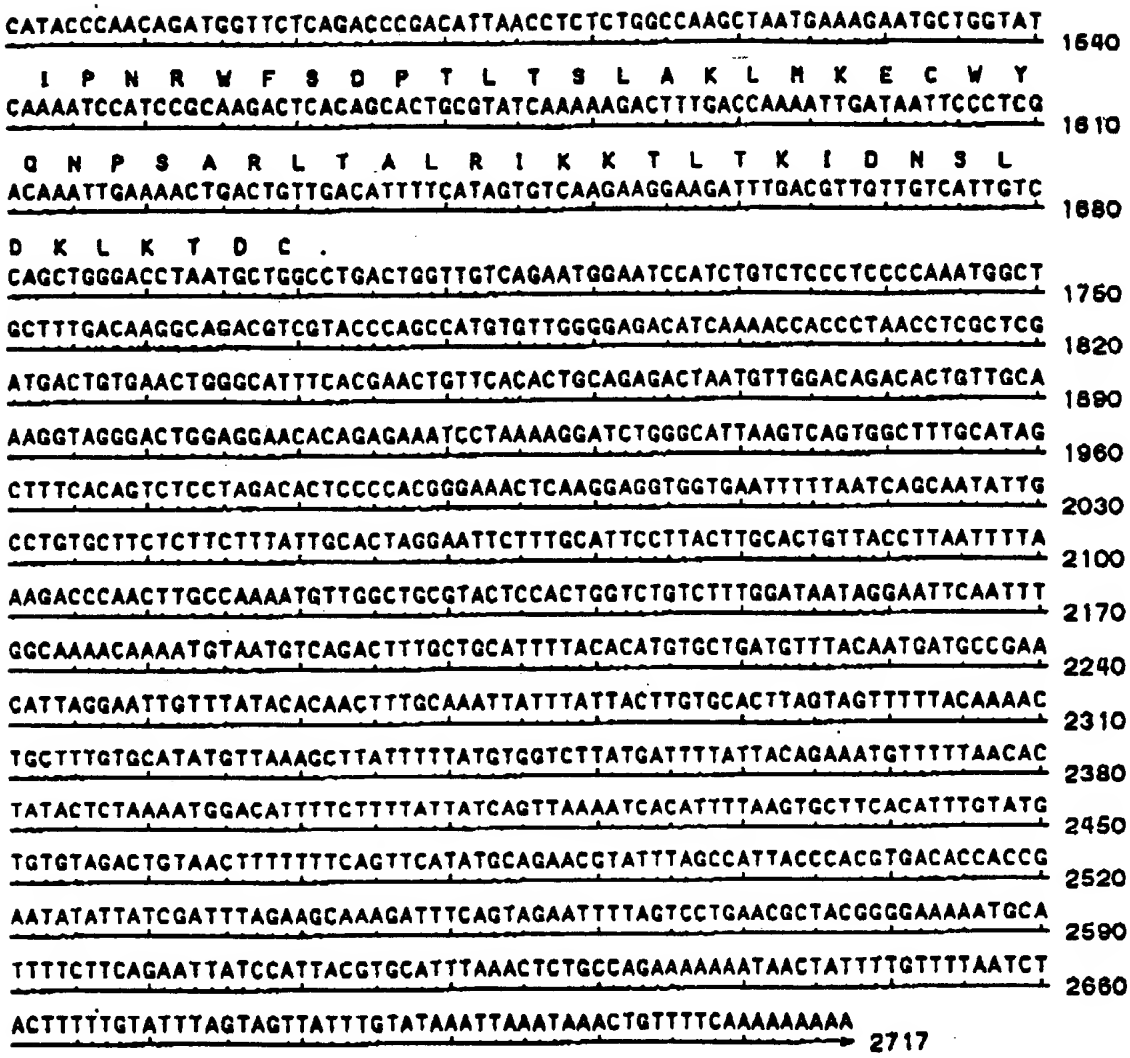
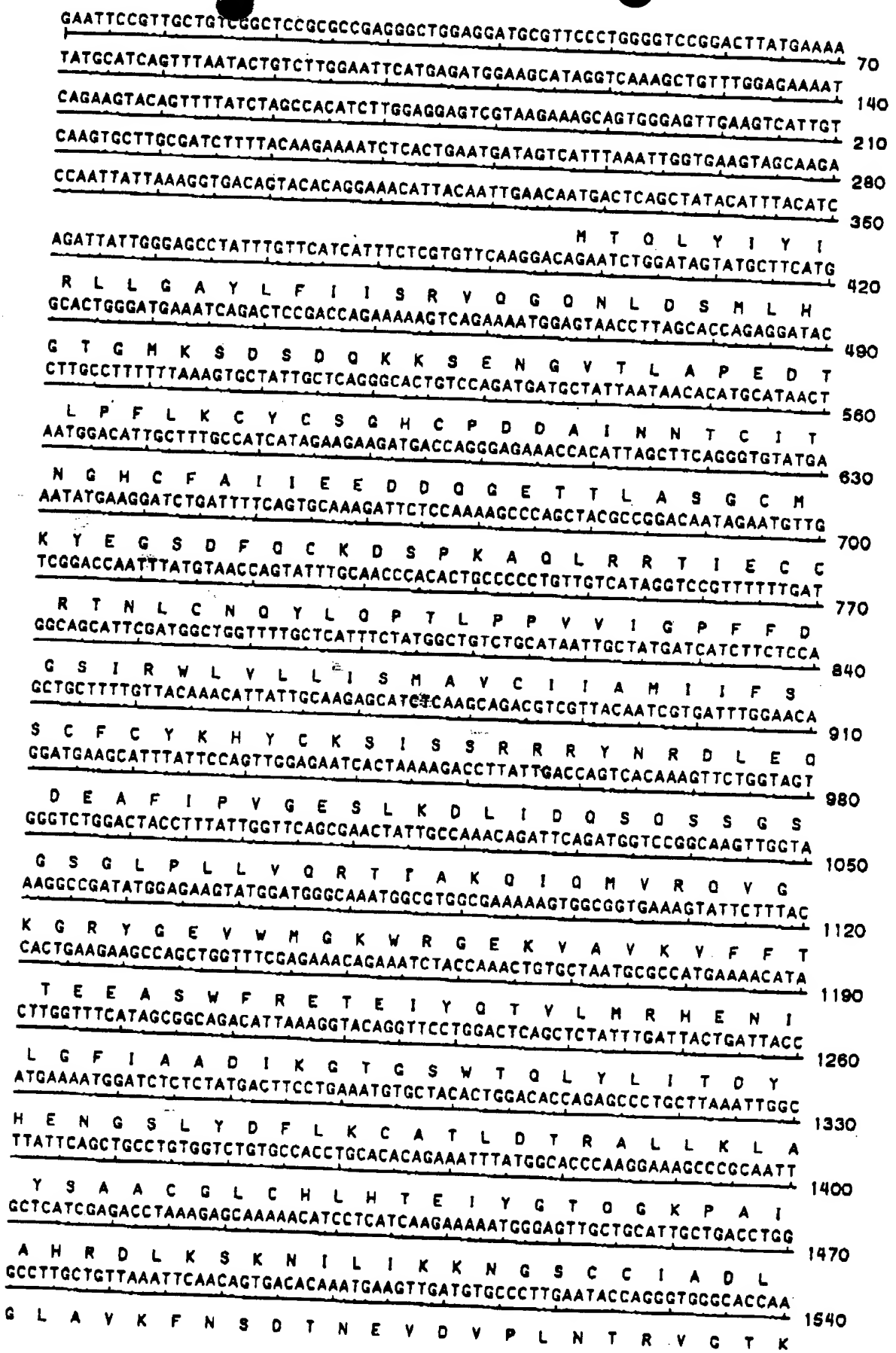
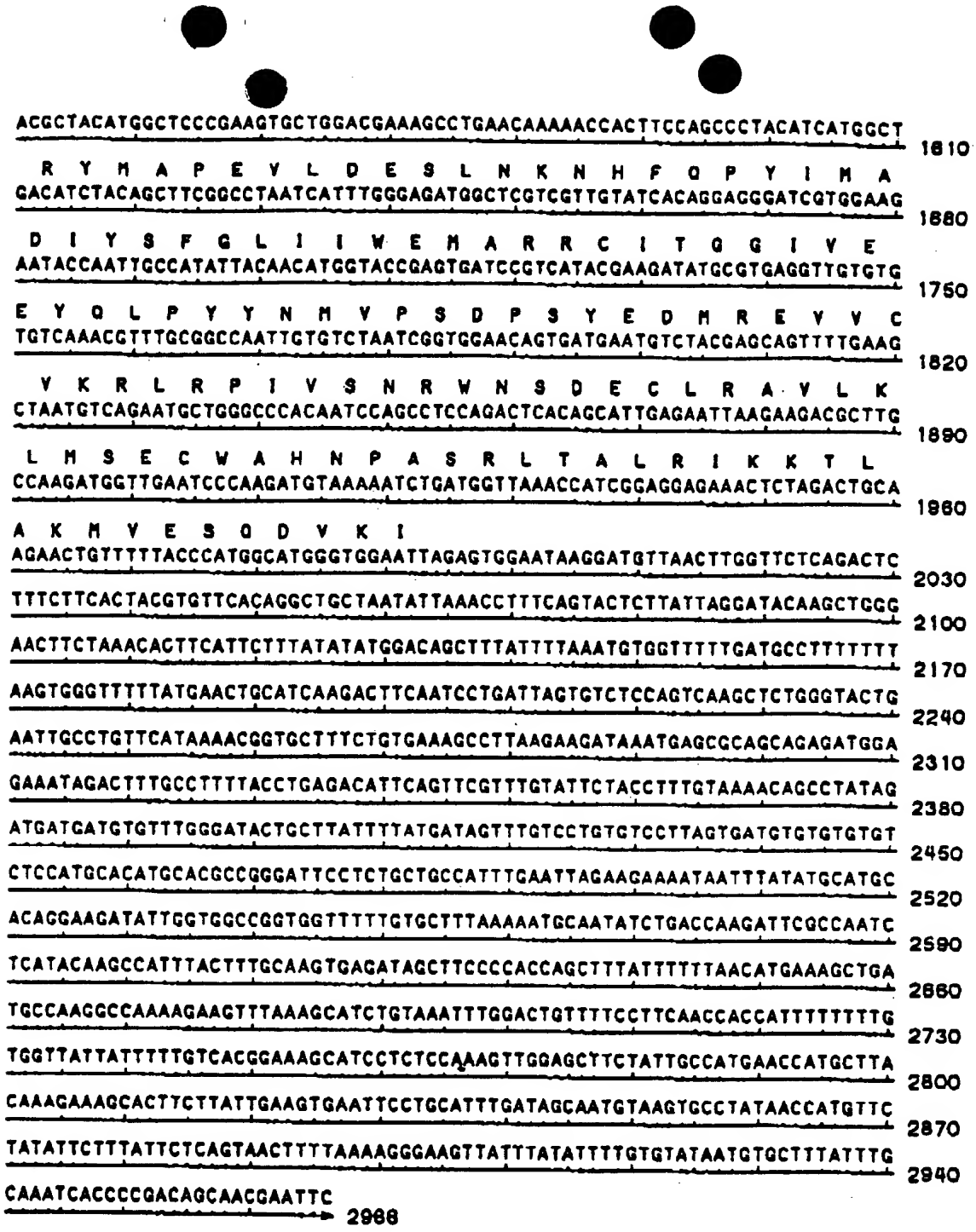



Nucleotide sequence and deduced amino acid sequence of activin receptor related kinase - 2 (ARK-2). The complete sequence of cDNA clone HP 53 is shown, which was obtained by screening a human placenta cDNA library with PCR product 11.2 as a probe.

ARK-3 Sequence Figure 8

(4)

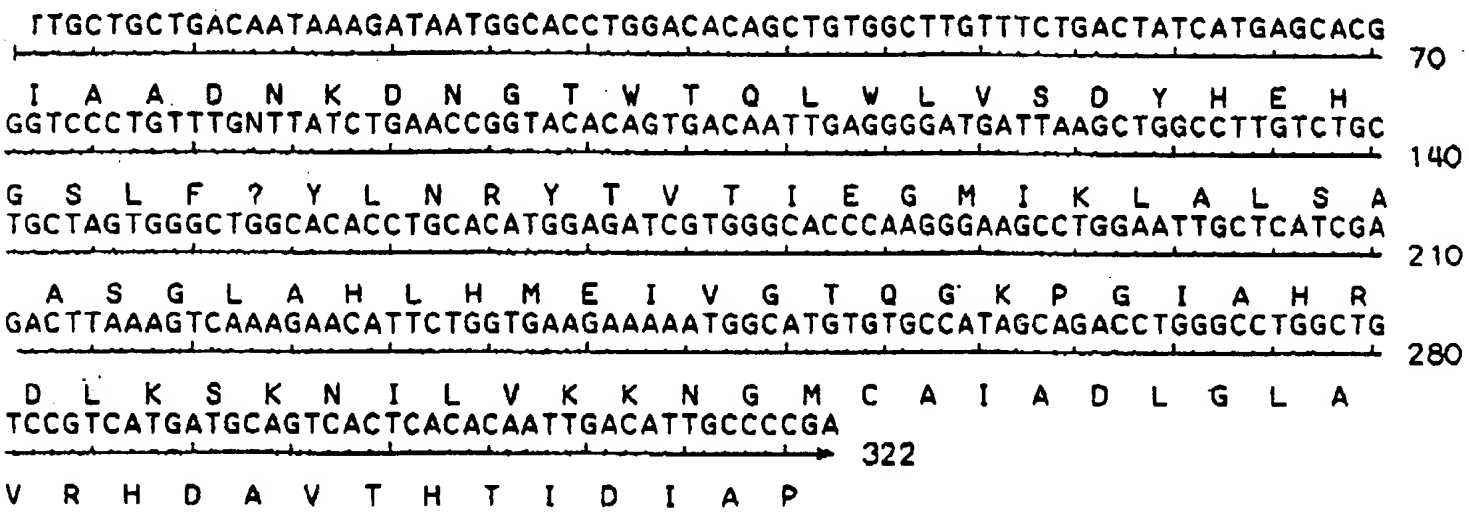





Nucleotide sequence and deduced amino acid sequence of activin receptor related kinase - 3 (ARK-3). The sequence of cDNA clone ONF5 is shown, which was obtained by screening a human foreskin fibroblast cDNA library with the kinase domain of cDNA clone ON11 as a probe. (cDNA clone ON11 was obtained from human fibroblast cDNA library using oligonucleotide derived from PCR product 5.1 as a probe)

ARX-4 Figure 1

(8)

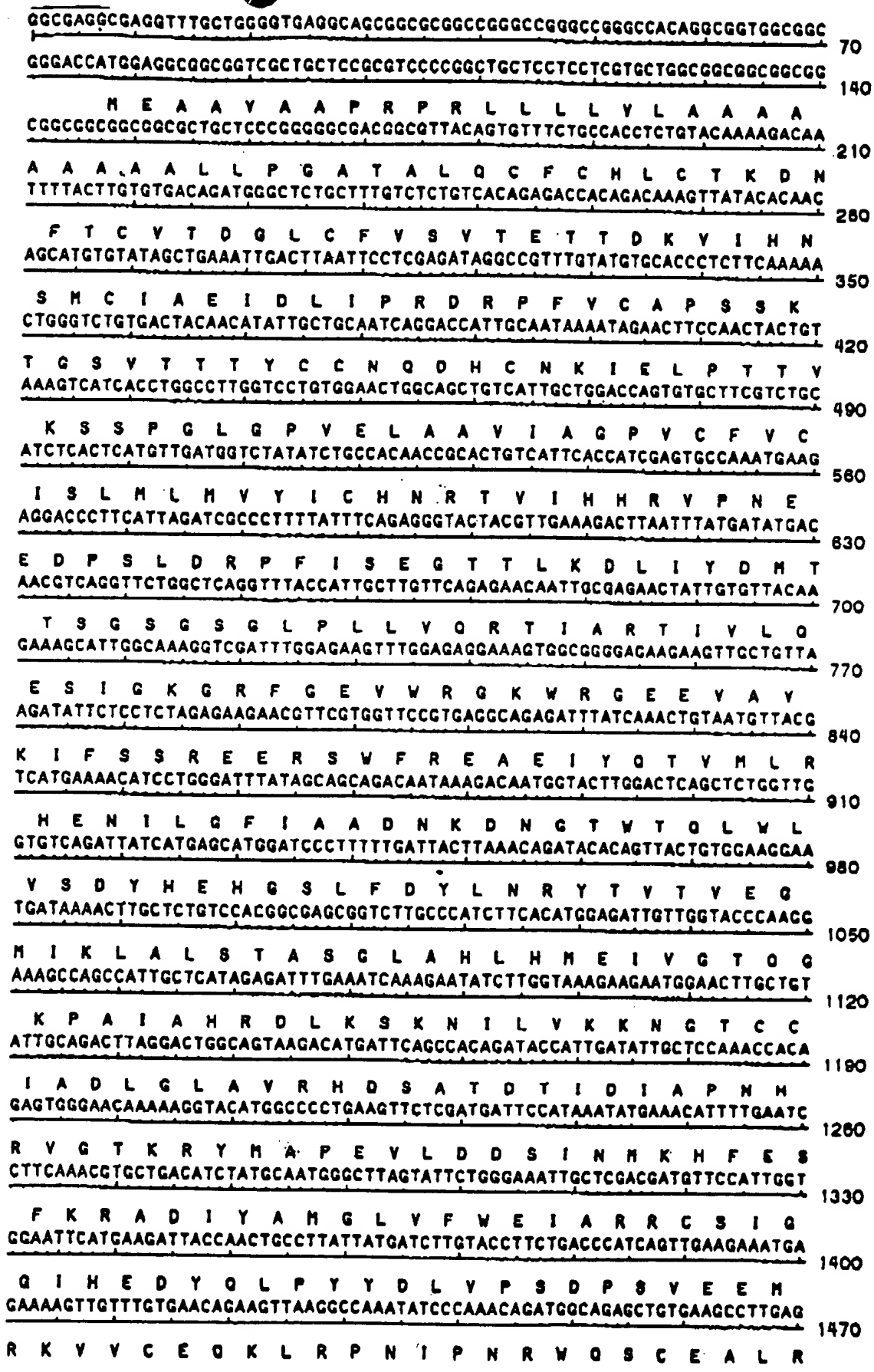



Partial nucleotide sequence and deduced amino acid sequence of a PCR product 11.1. This PCR product was obtained using human erythroid leukemia cells as mRNA source in the cDNA preparation and primers B3-S and E8-AS in the PCR.

ARK-5 Sequence

Figure 10





```
AGTAATGGCTAAAATTATGAGAGAATGTTGGTATGCCAATGGAGCAGCTAGGCTTACAGCATTGCGGATT                1540
  Y  M  A  K  I  M  R  E  C  W  Y  A  N  G  A  A  R  L  T  A  L  R  I
AAGAAAACATTATCGCAACTCAGTCAACAGGAAGGCATCAAAATGTAATTCTACAGCTTTGCCTGAACTC                1610
  K  K  T  L  S  Q  L  S  Q  Q  E  G  I  K  M  .
TCCTTTTTTCTTCAGATCTGCTCCTGGGTTTTAATTTGGGAGGTCAGTTGTTCTACCTCACTGAGAGGGA                1680
ACAGAAGGATATTGCTTCCTTTTGCAGCAGTGTAATAAAGTCAATTAAAAACTTCCCAGGATTTCTTTGG                1750
ACCCAGGAAACAGCCATGTGGGTCCTTTCTGTGCACTATGAACGCTTCTTTCCCAGGACAGAAAATGTGT                1820
AGTCTACCTTTATTTTTTATTAACAAAACTTGTTTTTTAAAAAGATGATTGCTGGTCTTAACTTTAGGTA                1890
ACTCTGCTGTGCTGGAGATCATCTTTAAGGGCAAAGGAGTTGGATTGCTGAATTACAATGAAACATGTCT                1960
TATTACTAAAGAAAGTGATTTACTCCTGGTTAGTACATTCTCAGAGGATTCTGAACCACTAGAGTTTCCT                2030
TGATTCAGACTTTGAATGTACTGTTCTATAGTTTTTCAGGATCTTAAAACTAACACTTATAAAACTCTTA                2100
TCTTGAGTCTAAAAATGACCTCATATAGTAGTGAGGAACATAATTCATGCAATTGTATTTTGTATACTAT                2170
TATTGTTCTTTCACTTATTCAGAACATTACATGCCTTCAAAATGGGATTGTACTATACCAGTAAGTGCCA                2240
CTTCTGTGTCTTTCTAATGGAAATGAGTAGAATTGCTGAAAGTCTCTATGTTAAAACCTATAGTGTTT                2308
```

Nucleotide sequence and deduced amino acid sequence of activin receptor related kinase - 5 (ARK-5). The sequence of cDNA clone EMBLA is shown, which was obtained by screening a human HEL cDNA library with PCR products 11.1 and 11.2 as a probe.

Figure 11-

# COMPARISON OF KINASE DOMAINS

## percent similarity

| | ARK-1 | ARK-2 | ARK-3 | 11.1 PCR PRL/ARK-4 | ARK-5/KD | TGFBRII/KD | ActR-IIB/KD | ActR-II/KD | daf-1/KD | |
|---|---|---|---|---|---|---|---|---|---|---|
| ARK-1 | | 70.5 | 52.1 | 40.2 | 55.2 | 34.5 | 32.6 | 32.2 | 32.6 | 1 |
| ARK-2 | | | 45.1 | 59.8 | 65.5 | 35.1 | 37.9 | 34.4 | 36.2 | 2 |
| ARK-3 | | | | 61.7 | 65.5 | 33.8 | 38.3 | 33.7 | 35.2 | 3 |
| 11.1 PCR PRL/ARK-4 | | | | | 91.6 | 35.5 | 36.4 | 34.6 | 45.8 | 4 |
| ARK-5/KD | | | | | | 39.3 | 37.9 | 35.5 | 39.3 | 5 |
| TGFBRII/KD | | | | | | | 42.2 | 41.6 | 29.9 | 6 |
| ActR-II/KD | | | | | | | | 78.0 | 32.9 | 7 |
| ActR-IIB/KD | | | | | | | | | 27.1 | 8 |
| daf-1/KD | | | | | | | | | | 9 |

## dendrogram

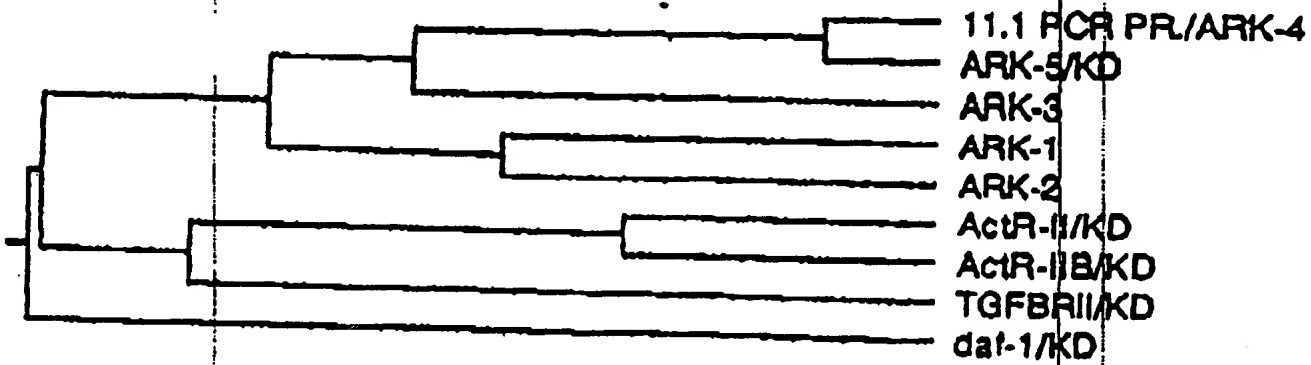



Figure 1

novel 5.1. Figure 12

(9)

GGATCCGAGACATTAAGAGTAACAATGTGTTGATTAATACCTACAGTGGTGTTCTCAAGATCTCTGACTT 70

R I R D I K S N N V L I N T Y S G V L K I S D F

CGGAACATCAAAGAGGCTTGCTGGCATAAACCCCTGTACTGAAACTTTTACTGGTACCCTCCAGTATATG 140

G T S K R L A G I N P C T E T F T G T L Q Y M

GCCCCCGAATTC 152

A P E F

Nucleotide sequence and deduced amino acid sequence of a PCR product 5.1. This PCR product was obtained using human erythroid leukemia cells as mRNA source in the cDNA preparation and primers B7-S and E8-AS in the PCR. The primer sequences are included ; these sequences, in particular sequence forming the restriction sites, may not correspond with the gene sequence from which the PCR product was derived.

Neural (3.1) Figure 13

(10)


Partial nucleotide sequence and deduced amino acid sequence of a PCR product 3.1. This PCR product was obtained using human erythroid leukemia cells as mRNA source in the cDNA preparation and primers B7-S and E8-AS in the PCR. The primer sequence is included ; this sequence, in particular the sequence forming the restriction site, may not correspond with the gene sequence from which the PCR product was derived.

Novel 112-6 Figure 14

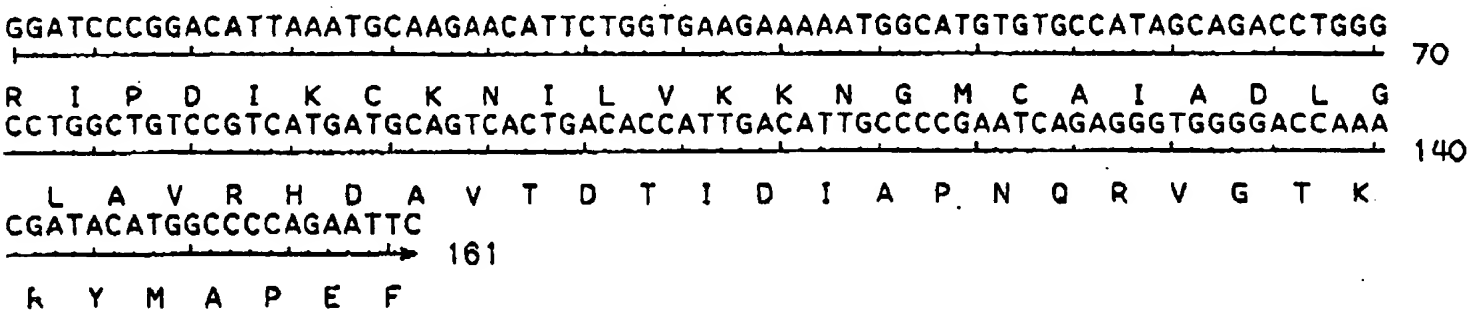
(ii)

```
GGATCCGCTGACATCAAGACCTAAGAACATCATCGTCAGCAGCCAGGCCCACATCAAACTGACCGACTTT
                                                                        70
  D  P  L  T  S  R  P  K  N  I  I  V  S  S  Q  A  H  I  K  L  T  D  F
GGACTCTGCAAGGAGTCTATCCATGAGGGCGCCGTCACTCACACCTTCTGCGGCACCATTGAGTACATGG
                                                                       140
  G  L  C  K  E  S  I  H  E  G  A  V  T  H  T  F  C  G  T  I  E  Y  M
CCCCTGAATTC
          151
A  P  E  F
```

Nucleotide sequence and deduced amino acid sequence of a PCR product 1/2-6. This PCR product was obtained using human erythroid leukemia cells as mRNA source in the cDNA preparation and primers B7-S and E8-AS in the PCR. The primer sequences are included ; these sequences, in particular sequence forming the restriction sites, may not correspond with the gene sequence from which the PCR product was derived.

Novel 1/2-7 Figure 15

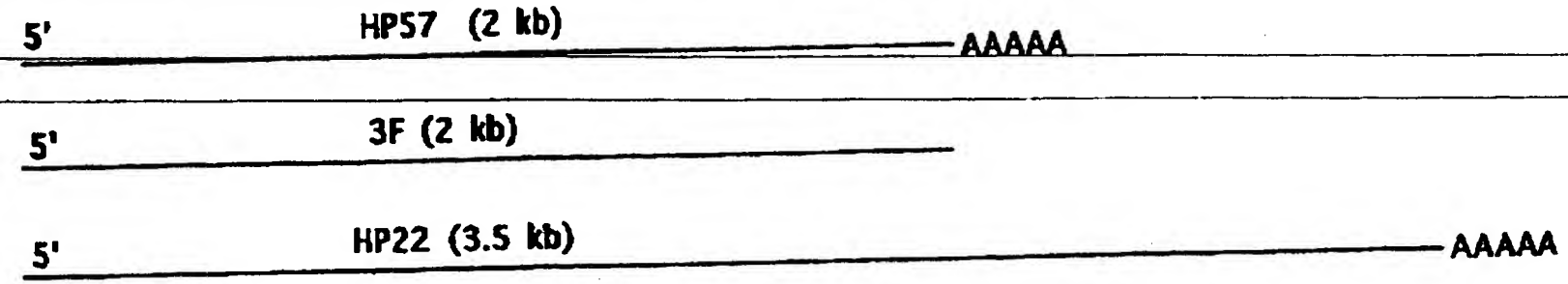
(12)

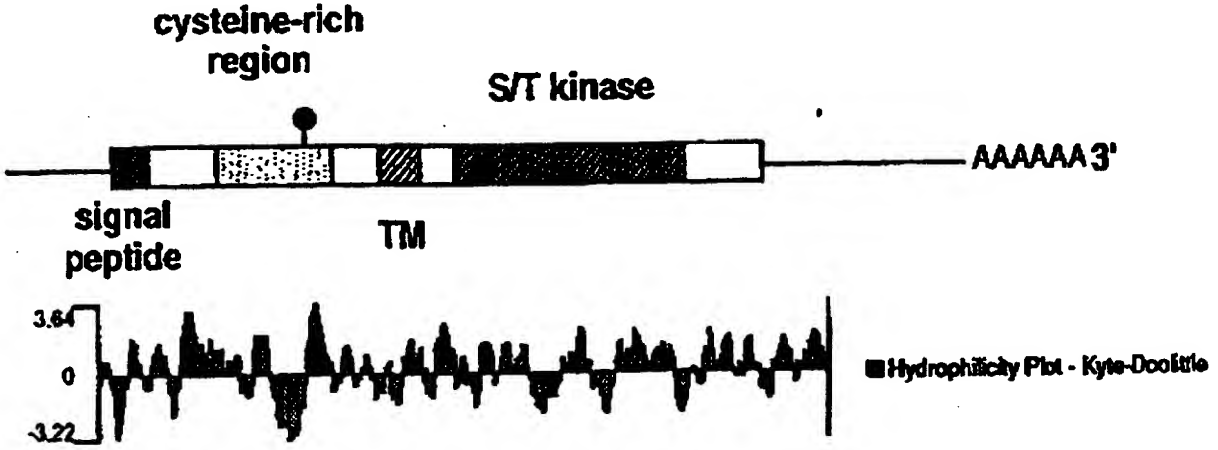



Nucleotide sequence and deduced amino acid sequence of a PCR product 1/2-7. This PCR product was obtained using human erythroid leukemia cells as mRNA source in the cDNA preparation and primers B7-S and E8-AS in the PCR. The primer sequences are included ; these sequences, in particular sequence forming the restriction sites, may not correspond with the gene sequence from which the PCR product was derived.

# ARK-1

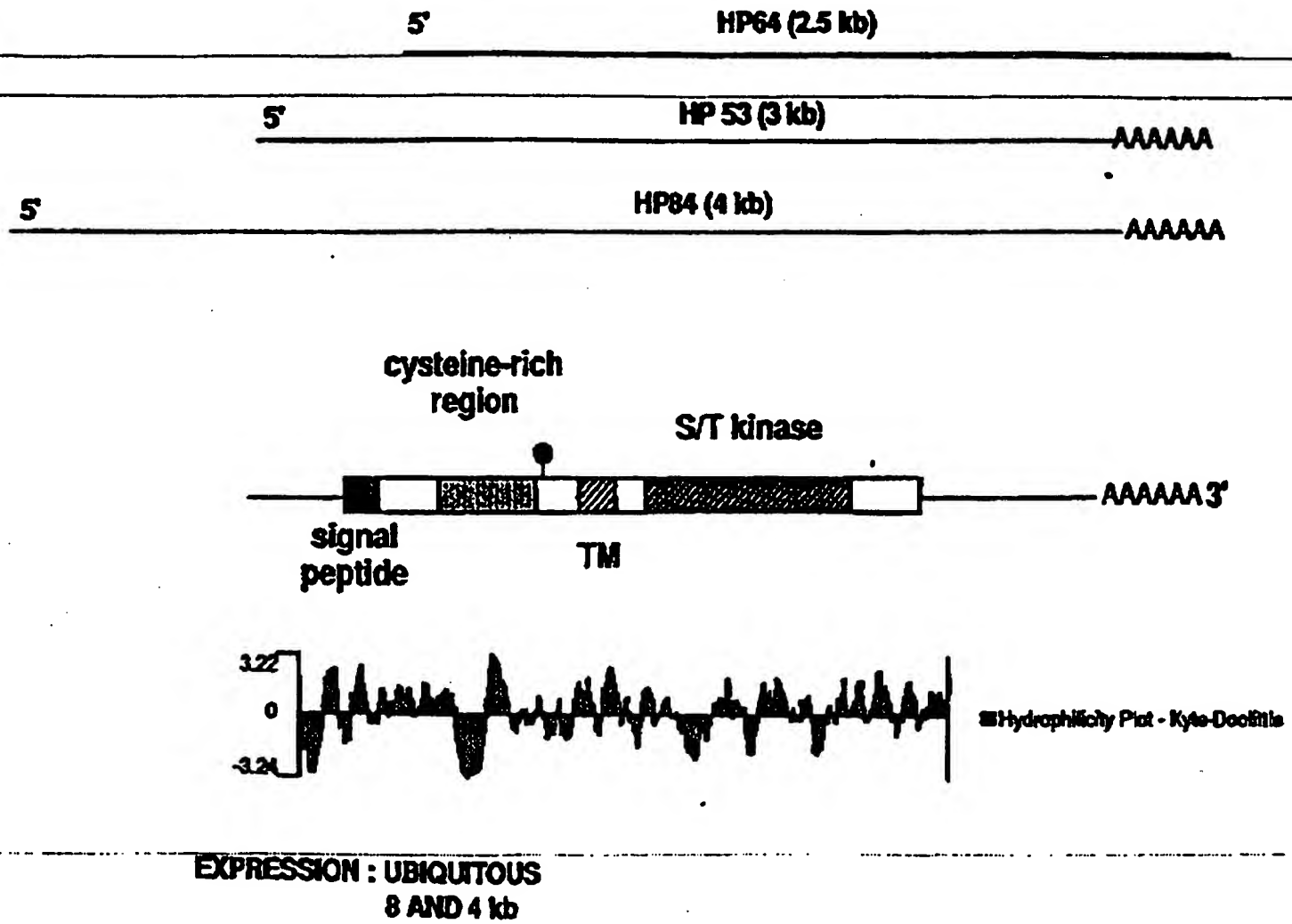
Figure 1B



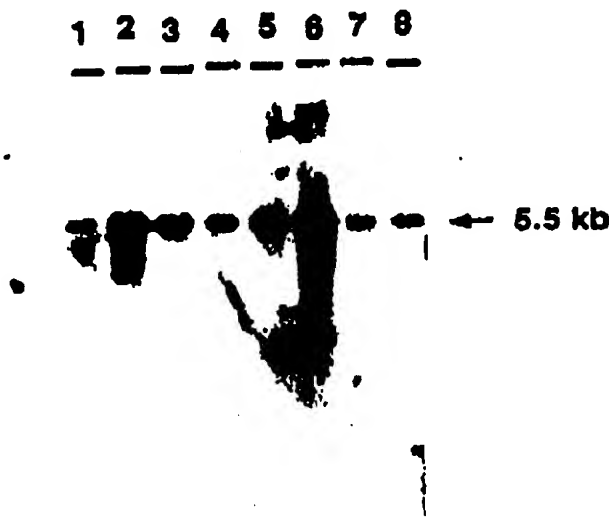

EXPRESSION : RESTRICTED
HUMAN FIBROBLASTS
5 AND 1.8 KB

# ARK-2




EXPRESSION : UBIQUITOUS
8 AND 4 kb

Figure 22




Northern blot analysis
mRNA (2 microgram) from different human tissues (Clontech, CA, USA) was hybridized with a radiolabeled 1 kb EcoRI fragment of cDNA clone EMBLA, corresponding to ARK-5, in the presence of 50 % formamide and 5 x SSC. Filter was washed with 0.5 x SSC, 0.1 % SDS at 55 C. Lane 1, heart; lane 2, brain; lane 3, placenta; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; lane 8, pancreas.